THE UNIVERSITY OF ALBERTA

ALLOPURINOL AND AZATHIOPRINE THERAPY OF CANINE RENAL ALLOGRAFTS

by

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A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "ALLOPURINOL AND AZATHIOPRINE THERAPY OF CANINE RENAL ALLOGRAFTS", submitted by John L. Bishop in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Imuran (azathioprine) is metabolized to 6-mercaptopurine which then enters into the pathways of purine metabolism, or is converted into an inactive product, thiouric acid, and excreted. Conversion to thiouric acid is catalyzed by xanthine oxidase.

A xanthine oxidase inhibitor, allopurinol, has been shown to potentiate the antitumor effect of 6-mercaptopurine in mice.[25] A combination of the two drugs has also prolonged canine renal allograft survival in two of three dogs.[74] No interactions between azathioprine and allopurinol have been shown experimentally.

A series of canine renal allografts were undertaken in which it was shown that there was an interaction between allopurinol and azathioprine in immunosuppression, probably due to inhibition by allopurinol degradation of azathioprine. However, leucotoxicity was greater with the particular combination of drugs used, when compared to azathioprine alone, and it was concluded that this particular dose combination probably would not offer any advantage in clinical use over current immunosuppressive regimes.

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HISTORICAL REVIEW

Modern transplantation began with the development of techniques of transplantation by Ullman and Carrel in 1902.[1,6] They did not differentiate between autografts and allografts, ascribing all of their failures to technical difficulties.

While others speculated that biological as well as technical factors existed, the immunologic nature of graft rejection was not clearly shown until the classic studies of Medawar from 1944 to 1946 in which he studied skin grafts in the rabbit, showing that a second skin graft taken from a given donor and placed on the same recipient as the first graft underwent accelerated rejection, whereas a graft from a third individual to the original recipient was rejected at the same rate as the first graft.[2-5]

Clinical renal transplantation seems to have begun in 1948, when Hufnagel, Hume and Landsteiner transplanted a kidney to the anticubital fossa of a woman with reversible acute tubular recrosis with three day graft function.[7] From 1950 to 1953, fifteen allografts were transplanted without immunosuppression,[7-9] the last six being placed in the iliac fossa using a technique developed by Murray.[10] Most rejected within a few weeks, but one survived 5 ½ months. Though the results were discouraging, a definite

technical advance had been made which justified later transplantation.

Harrison, Merrill and Murray of the Peter Bent Brigham Hospital in Boston successfully transplanted a kidney from an identical twin in 1954.[11] The first long-term success in a transplant between non-identical twins was in 1959.[7]

Following the introduction of suitable techniques for renal transplantation, and the recognition of the immunologic nature of graft rejection, the major problem in clinical transplantation has been immunosuppression. Murray avoided the immune reaction in 1954 by using identical twins. Total body irradiation was introduced in 1958,[1] and resulted in the first long-term success between non-identical twins in 1959.[7] However, the balance between graft rejection and patient survival was too precarious and this method was short lived. In 1959, Schwartz and Dameshek reported that 6-mercaptopurine depressed the immune response to serum proteins and prolonged skin graft survival in the rabbit.[12,13] Calne then showed that this drug would prolong renal allograft survival in the dog.[14-16] Shortly after, he showed that an analogue of 6-MP, azathioprine, also prolonged dog renal allograft survival with an apparently lesser amount of bone marrow toxicity.[6,15] Azathioprine then became widely used clinically and has

been the basic immunosuppressive drug for almost all transplantation.

In addition to azathioprine, Prednisone is used in almost all clinical transplants. Other agents of immunosuppression or methods of minimizing the immune response that are in common clinical use are local graft irradiation, Actinomycin C, ALS and histocompatibility typing.[17]

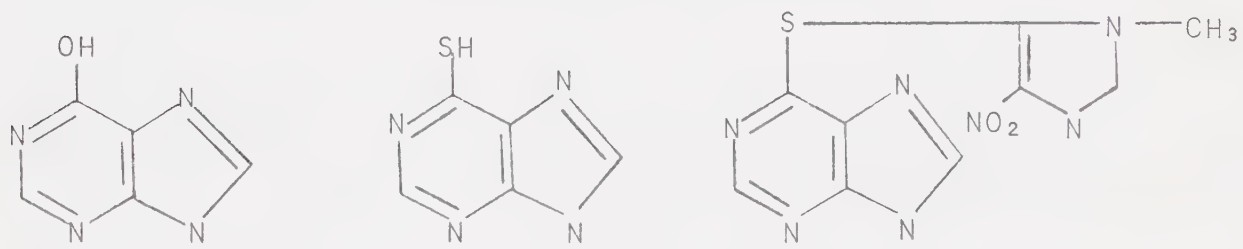
In Calne's experiments with azathioprine, deaths due to toxicity, mainly of bone marrow, occurred in almost 50% of the experimental animals. Clinically, initial use of azathioprine at a dose of 3-15 mg./kg. was accompanied by severe bone marrow depression and 40% mortality from infection in the first three months.[18] With the development of various techniques and the use of auxiliary drugs which eliminated the need for high doses of azathioprine, the early mortality dropped.[22,23] Present practice is to use the maximum tolerated dose as measured by development of leukopenia. This dose is usually 2-3 mg./kg./day and is almost always given with Prednisone in various amounts.[7] Though acute toxicity with azathioprine has become infrequent, the major cause of patient death with kidney transplantation has been drug toxicity and/or infection without rejection,[18-24] however it must be remembered that these patients are not receiving azathioprine only.

Theoretically, one might lessen the toxic effects

of an agent by using it in combination with a protective agent which counteracts its toxicity more than its therapeutic effect. Elion has stated that the use of allopurinol in combination with 6-mercaptopurine in mice results in a better therapeutic index of 6-mercaptopurine as an anti-tumor agent due to a four-fold increase in anti-tumor activity as compared to a two and one-half times increase in toxicity.[25] She was also able to show that allopurinol potentiated the immunosuppressive action of 6-MP in inhibiting the formation of antibody to sheep red blood cells. As azathioprine is an analogue of 6-mercapto-purine, an experimental series was undertaken to see if the use of allopurinol would potentiate the therapeutic (immunosuppressive) effect of azathioprine on canine renal allografts, and secondly whether any appreciable difference in bone marrow toxicity as compared to immunosuppressive effect would result.

AZATHIOPRINE METABOLISM

Azathioprine (BW 57-322), (Imuran)*, [6-(1-methyl-4-nitro imidazole-5-ylthio) purine], is an imidazole substituted analogue of 6-mercaptopurine which in turn is a purine analogue. Azathioprine was synthesized by Hitchings and Elion[25] in hopes that the exidation of 6-mercaptopurine's -S atom would be reduced and therefore slower metabolic degradation and a more prolonged therapeutic action would result.



hypoxanthine 6-mercaptopurine Azathioprine

Absorption

Bach[27] states that within 15 minutes of oral administration of S^{35} labelled azathioprine measurable serum radio-activity is present, with a peak being reached in 30 minutes. Rossette inhibiting activity of serum in man appears within one-half hour and reaches a maximum within one hour of oral or intravenous azathioprine.

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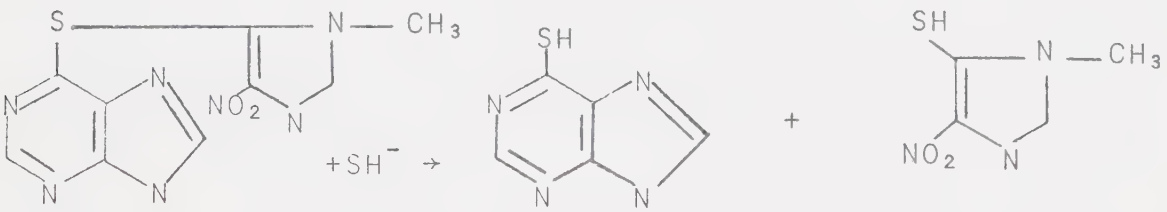
*Burroughs Wellcome & Co.

Metabolic Activation

Azathioprine is split in vitro[28-30] and in vivo[31] by sulfhydryl groups to form 6-mercaptopurine and a nitro-imidazole moiety. The fate of the imidazole is not presently known, though there is speculation that it has an immunosuppressive action of its own, probably by binding sulfhydryl groups on proteins at the time of splitting of azathioprine. 6-MP is in turn converted to thioinosinic acid,[32,33] a 6-MP ribotide, via competition with hypoxanthine for inosinic acid pyrophosphorylase.[34]



Azathioprine 6-MP nitro-imidazole

Thioinosinic acid has been shown to act via "pseudo feedback inhibition"[35] and substrate competition[32,36-42] on the first and last steps respectively of biosynthesis of the purine nucleotides of adenine and guanine as shown in figure 1. Feedback inhibition is thought to be the most important biochemical effect in vivo.[43] Guanylic and adenylic acid are important as components of RNA, DNA, and various coenzymes. Inhibition of nucleic acid synthesis has been shown by measuring total DNA[44] and incorporation of labelled precursors into nucleic acids.[45-47]

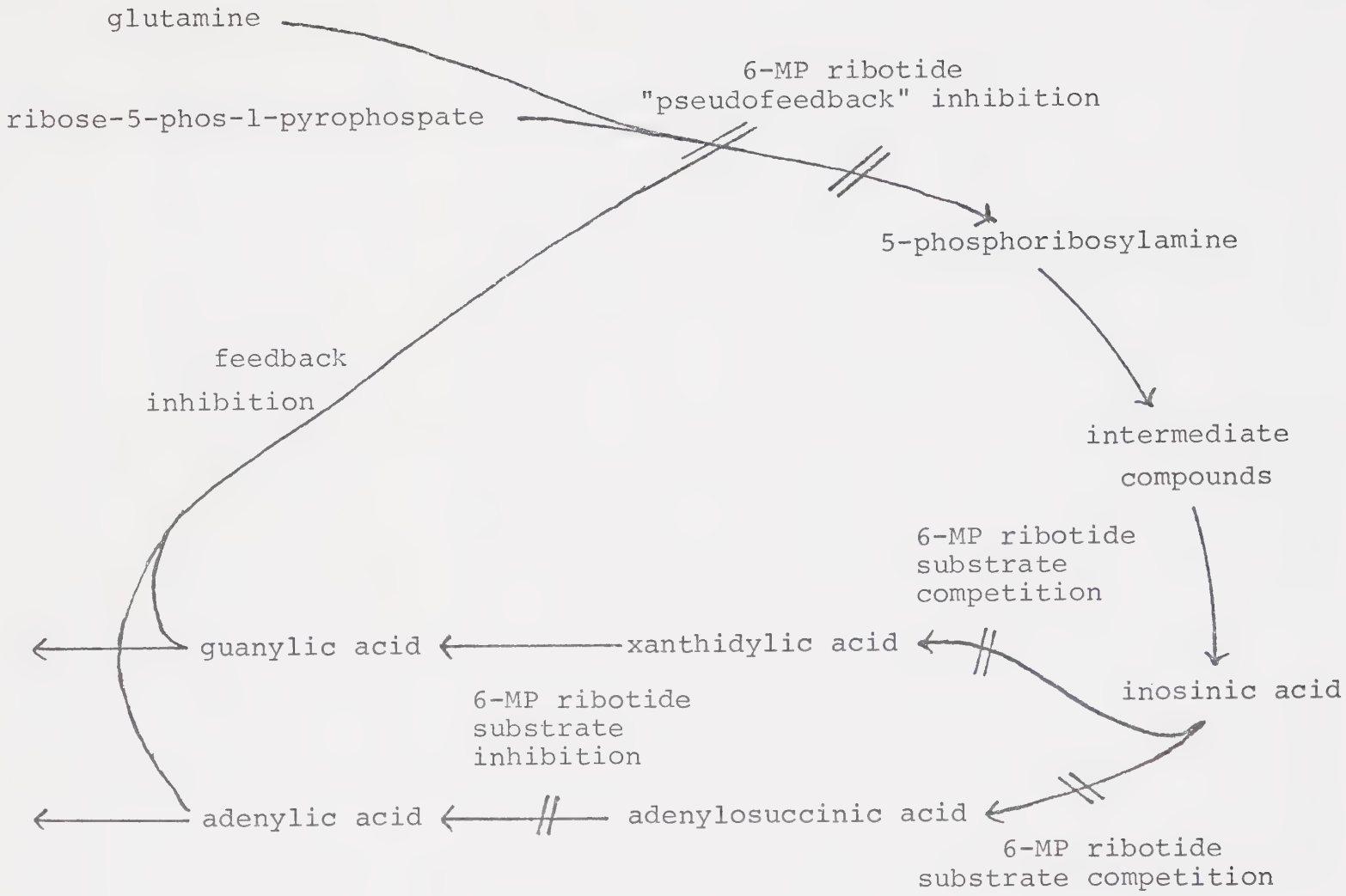


Figure 1 - Principle Sites of Action of 6-Mercaptopurine on Purine Metabolism. After Berenbaum[83]

In addition, the triphosphate of thioinosine has been shown to inhibit the formation of coenzyme NAD[48] and to interfere with the acetylating function of coenzyme A.[49]

Overall it would seem that thiopurines might inhibit DNA and RNA synthesis, both early in de novo synthesis and at the purine nucleotide interconversion steps; they might therefore be incorporated into nucleic acids causing possible malfunction of a particular form of nucleic acids; they can inhibit coenzyme formation and function and therefore might interfere with the various aspects of cell metabolism. However, Elion[50] has stressed that while the biochemical actions are known, the significance of these actions (as outlined in the preceding sentence) are deduction only, and in particular, explanation of the action of the purine analogues on the immune reaction is speculative.

Degradation

Degradation of 6-mercaptopurine occurs within four hours of azathioprine intake.[27] The principle pathways are summarized in figure 2.

All of the derivatives shown in figure 2 except 6-methylthiopurine (6-SCH_3) have been found in the urine of patients taking 6-mercaptopurine.[51]

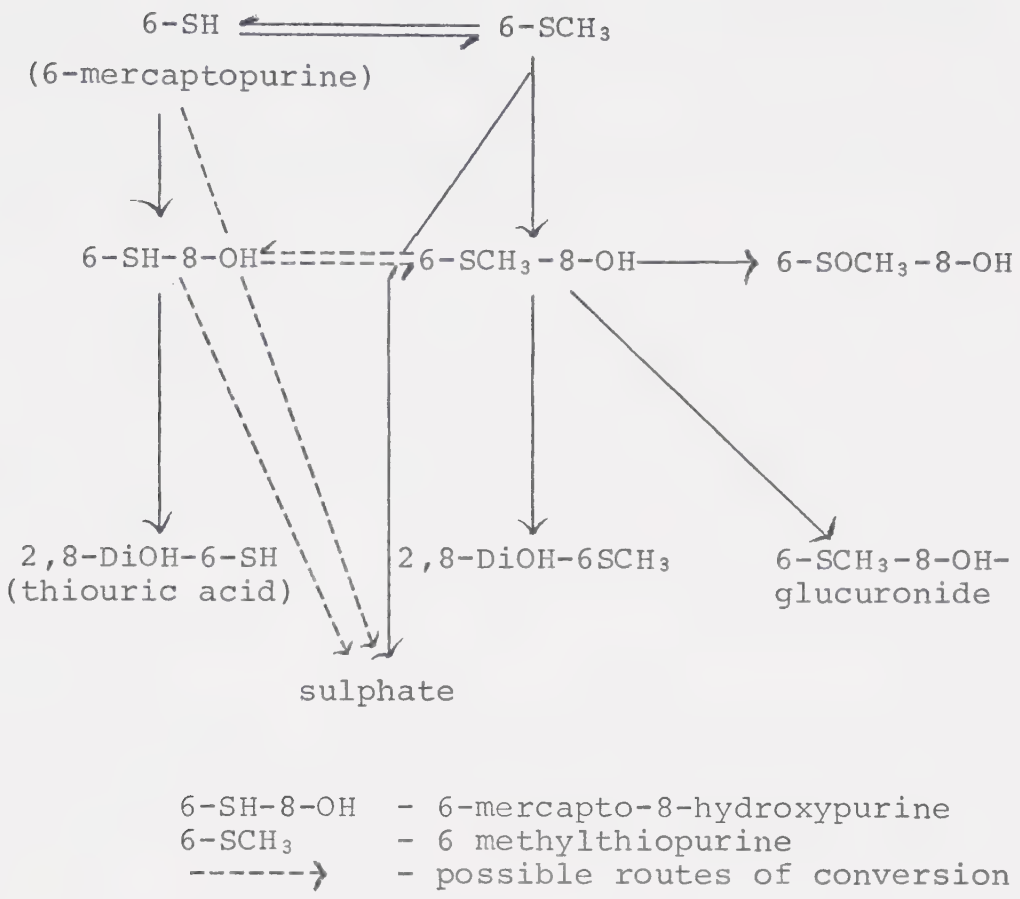


Figure 2 - Principle Pathways of 6-Mercaptopurine Degradation in vivo. After Elion[50]

There are two main pathways of 6-mercaptopurine catabolism:

1) direct oxidation by xanthine oxidase to thiouric acid, probably via 6-mercapto-8-hydroxypurine,[50]

and 2) methylation of the sulphur and similar (to 1) oxidation reactions of the methylated derivatives.

Bach has suggested that other enzymes as well as xanthine oxidase may be involved, as he has studied a

patient with gout who showed a hereditary, and probably enzymatic, lack of degradation.[27]

The principle site of catabolism is thought to be the liver.[51,52]

A large amount of inorganic sulphate is also formed and is detectable in the urine of patients receiving 6-mercaptopurine.[50] In man, this is not affected by xanthine oxidase inhibition.[50]

Elimination

6-mercaptopurine and its metabolites are eliminated by the kidneys.[50]

Elion has shown that after 6-mercaptopurine 125 mg. p.o. in man free 6-mercaptopurine appears in the urine in maximal amounts at 4 to 6 hours and persists up to 10 to 12 hours, as do the metabolites thiouric acid, 6-methylsulphinyl-8-hydroypurine and inorganic sulphate. A total of 46% of the oral dose could be accounted for in the urine in the first six hours.[50]

After 150 mg. 6-mercaptopurine p.o. in man, Elion reports that 7.0% appeared as free 6-mercaptopurine and 25.5% as thiouric acid in the urine in the first twelve hours.[25] No free 6-MP and less than 1% thiouric acid

appeared in the second twelve hours. Vogler, in a study of six leukemic patients receiving 25 to 100 mg. of 6-mercaptopurine p.o., reports that less than 3% of the original dose appears as free urinary 6-mercaptopurine. Almost all appeared in the first six hours.[53]

Similar results have been obtained with azathioprine. In chronic granulocytic leukemia patients on azathioprine therapy, after an oral dose of 150 to 300 mg. p.o., excretion as 6-mercaptopurine ranges from 1.3 to 2.4%, as thiouric acid from 13.0 to 21.4% and as azathioprine from 0 to 2.25% in the first twenty-four hours.[30] In transplanted dogs with good renal function, urinary 6-MP ranges from 0.8 to 3.6% of the oral dose in the first twenty-four hours, thiouric acid ranges from 13.7 to 28.0% and azathioprine from 6.6 to 11.8%.[30]

Toxicity

Leucopenia has been a frequent experimental occurrence in dogs receiving azathioprine. For instance, Starzl noted bone marrow depression in three out of eighteen dogs receiving 2-4 mg./kg./day.[54] Calne recorded bone marrow toxicity in four out of nineteen dogs.[6] Clinically, leucopenia is a major problem and usually is the factor that determines the dose of azathioprine that may be used. Patient tolerance of azathioprine is variable, and if it is

low may prevent the administration of a dose sufficient to prevent rejection. A recent report indicates that splenectomy permits higher doses of azathioprine, and that this may be useful to those renal transplant patients in whom leucopenia prevents administration of adequate doses for immunosuppression.[55]

Infection has been more frequent, occurring in twelve of the nineteen dogs in Calne's series.[6] Clinically, it is the most prevalent complication and is responsible for most of the deaths occurring in transplant patients.[22-24] However, most transplant patients also receive Prednisone and other immunosuppressive treatment. In a study of twenty patients receiving 3 mg./kg./day of azathioprine only for assumed immune disease, infection was infrequent, occurring in only three patients.[56]

Azathioprine is markedly hepatotoxic in dogs[54,57] but in humans evidence for hepatotoxicity is infrequent.[58-60]

Finally, there is an increased incidence of malignancy, mainly of the lymphoreticular system, in patients receiving chronic immunosuppressive therapy.[61-63]

Azathioprine Metabolism and Disease

Clinically, it is an axiom that azathioprine dosage must be promptly reduced in the presence of impaired renal

function because of the increased danger of leucopenia and possibly fatal infection. The phenomenon of rejection with impaired renal function and immunosuppression leading to leucopenia and/or infection was frequently noted in the early experiences.[18,24] The recognition of these events and resulting policy of reducing the azathioprine dose with renal failure is felt to be an important factor in the reduction of mortality from acute toxicity and infection. The reason given for increased toxicity is that the elimination of azathioprine metabolites via the kidney is probably reduced, thus resulting in prolonged retention of the immunosuppressive.[64] This is not consistent with the studies of 6-mercaptopurine metabolism showing that only 3 to 7% of the original dose in a person with normal renal function is excreted in a metabolically active form (unmetabolized 6-MP).[25,53]

Secondly, J.F. Bach has studied fifty-nine patients given azathioprine for renal transplantation and did not find any difference in the incidence of leucopenia with or without renal failure. Also in fifteen non-transplant patients with renal failure, he found no modification of the rosette inhibition curve, but S^{35} (from labelled azathioprine) was retained.[65] He does not feel that the azathioprine dose should be reduced in the presence of renal failure.

Several variations from normal azathioprine metabolism have been found by J.F. Bach in patients with gout.[27] Azathioprine has failed to inhibit phytohemagglutinin-induced transformation in gouty patients with hypozanthine-guanine-phosphoribosyl-transferase. Bach showed that no rosette inhibition appeared in one patient with this abnormality. He also has studied two gouty patients who showed a marked bone marrow hypoplasia during the first fifteen days of treatment with azathioprine 3 mg./kg./day for renal transplantation. Both had a marked delay in disappearance of serum immunosuppressive activity as measured by rosette inhibition, as did the father and one brother of one of these patients.

Bach studied azathioprine metabolism (rosette inhibition, S^{35} azathioprine) in 18 patients with liver disease, finding that patients with severe disease due to cirrhosis or "cytolysis" had a complete absence of azathioprine activity while those with moderate disease showed a low level of activity with a delay in disappearance of activity in some cases.[27] Patients with obstructive jaundice showed no abnormality. Mitchell found that liver transplant patients with impaired liver function showed a lack of rosette inhibiting activity with azathioprine.[52]

ALLOPURINOL

Allopurinol [4-hydroxypyrazolo-(3,4-d)-pyrimidine] was first shown to be an effective xanthine oxidase inhibitor in vivo by Elion.[25] She showed that allopurinol inhibited degradation of 6-mercaptopurine in the mouse, and thereby potentiated its effectiveness as an antitumor and immunosuppressive agent. Following this, Rundles[66] and Yu[67] demonstrated its usefulness in the treatment of patients with gout, and it is now widely used in the treatment of this disease.

It has also been found that allopurinol affects de novo purine biosynthesis by the formation of allopurinol ribonucleotide from allopurinol and phosphoribosylpyrophosphate.[68,69] Allopurinol ribonucleotide is a known inhibitor of phosphoribosylpyrophosphate-amido-transferase.[71] Secondly, formation of the nucleotide has been shown to cause depletion of red blood cell phosphoribosylpyrophosphate in man.[72] The metabolic consequences, however, are unknown and are regarded as being probably minimal as no toxic effects of allopurinol have been described.[73]

In Elion's experiments,[25] she showed that allopurinol caused a two-thirds reduction in the minimally effective dose of 6-mercaptopurine that would inhibit antibody formation to sheep red blood cells. McConnell and Zukowski,

in an abstract, state that two of three dogs treated with allopurinol and 6-mercaptopurine for renal transplantation survived over thirty days.[74] It is these two papers that form the basis for our experiments.

METHODS

Experimental Groups

Thirty-four mongrel dogs of both sexes weighing 9 to 25 kilograms were used. Renal transplants were carried out in five experimental groups which varied according to the immunosuppressive therapy used:

Group 1 - Controls - no treatment given. 8 dogs

Group 2 - Imuran daily - Imuran 10mg./kg. p.o. on the day of surgery, then 5mg./kg. p.o. daily for three weeks, then 5mg./kg. p.o. twice weekly. 5 dogs

Group 3 - Imuran M.W.F. - Imuran 10mg./kg. p.o. on the day of surgery, then 10mg./kg. p.o. three times weekly (M.W.F., Monday, Wednesday, Friday). 7 dogs

Group 4 - Allopurinol - 200mg. p.o. on the day of surgery, then 100mg. t.i.d. daily. 6 dogs

Group 5 - Imuran and Allopurinol - Imuran given as in Group 3, Allopurinol as in Group 4 - i.e. Imuran 10mg./kg. p.o. and Allopurinol 200mg. p.o. on the day of surgery, then Allopurinol 100mg. t.i.d. daily and Imuran 10mg./kg. p.o. three times weekly (Monday, Wednesday, Friday). 8 dogs

Selection of Subjects

The dogs were "chronic" dogs obtained from the University Laboratory Animal Services where they had been

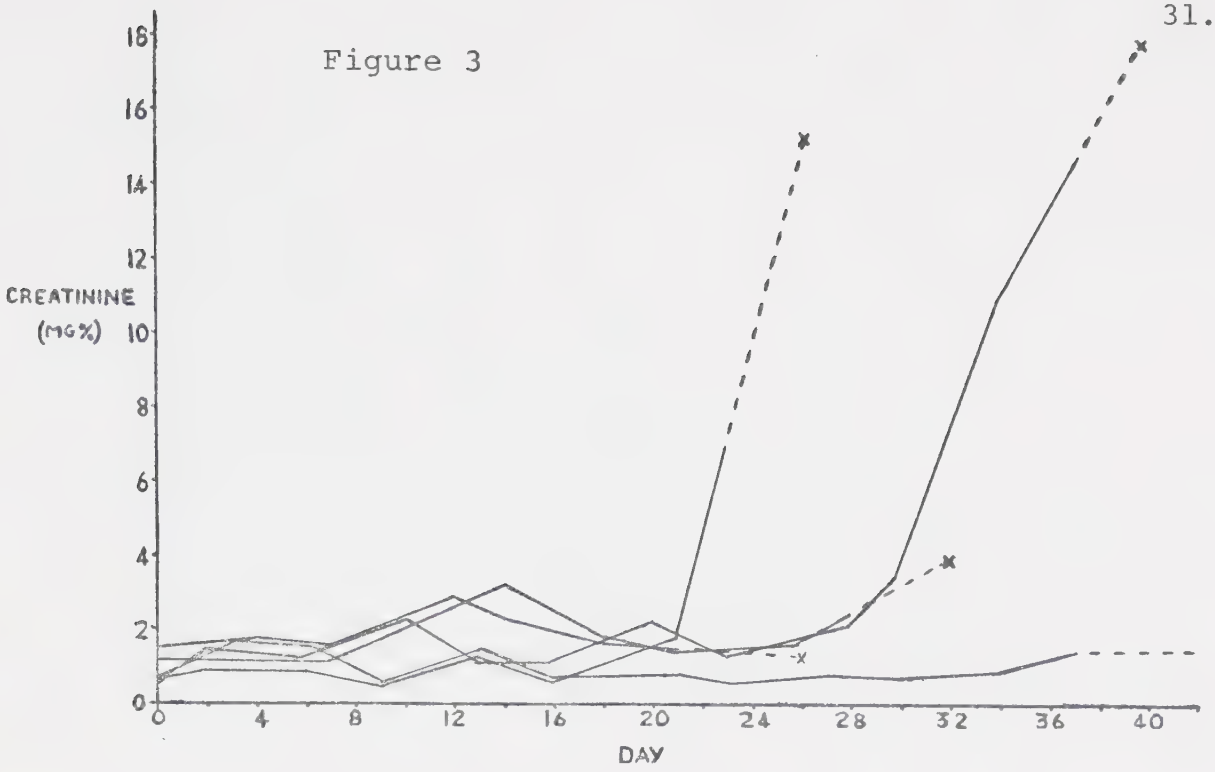
isolated and quarantined for two weeks, bathed and defleaed, vaccinated against infectious canine distemper and infectious canine hepatitis, and has a fecal analysis followed by deworming with the appropriate vermifuge or treatment with other appropriate antiparasitic agents if indicated.

Upon arrival at the Vivarium they were once again examined physically and unwell animals returned. The dogs, which usually arrived in groups of four to six, were paired for surgery by a surgical assistant on the basis of similarity in size and weight and dissimilarity in appearance. Assignment to the various treatment groups was made by the author without knowledge of which particular dogs were to be operated upon, and announced at the time of surgery. Reciprocal transplants were done within a pair, and both dogs of the pair received the same treatment.

Surgical Procedure

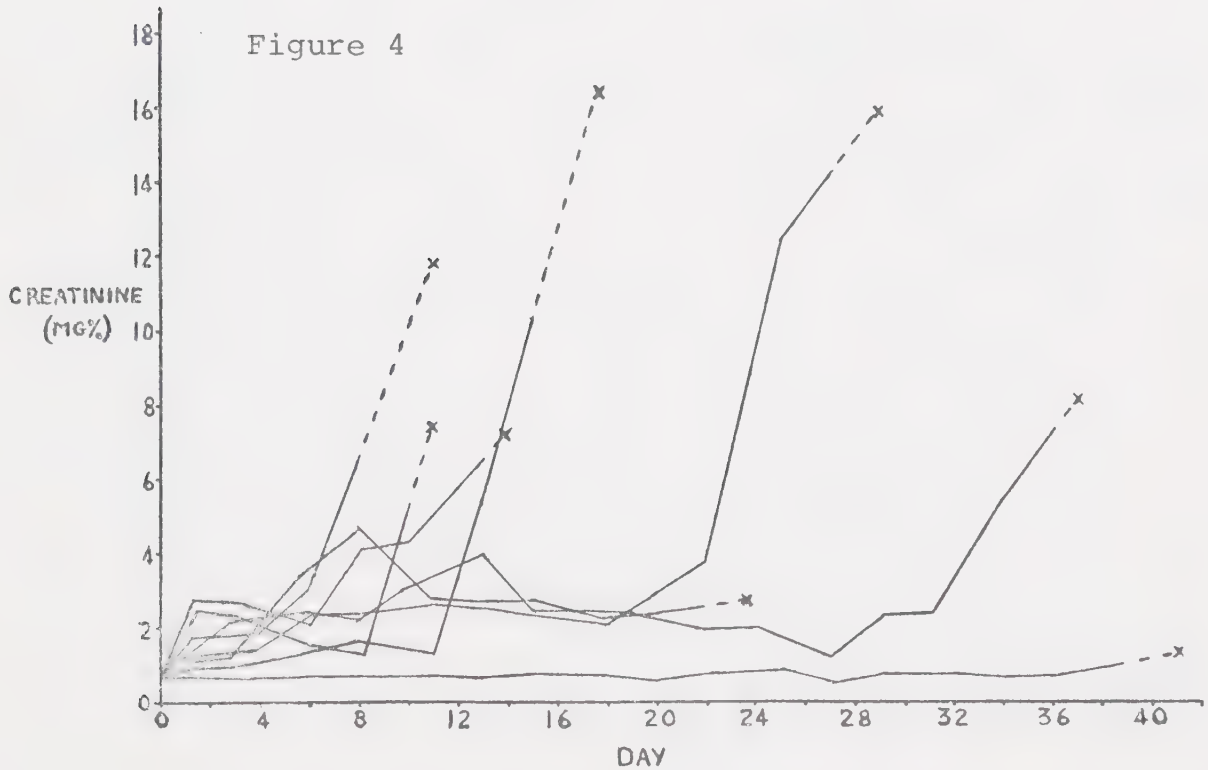
Diet on the day prior to surgery consisted of water ad libitum but no food. On the day of surgery the dogs were anesthetized with sodium pentobarbitol (Diabutal®) in an amount sufficient to induce moderate anesthesia (about 30mg./kg. B.W. i.v.), intubated and allowed to breathe spontaneously. Additional doses of 30 - 60 mg. were given as needed during the procedure. Each dog was given 150,000 I.U. of Benzathine Penicillin G. and 150,000 I.U. of

Figure 3



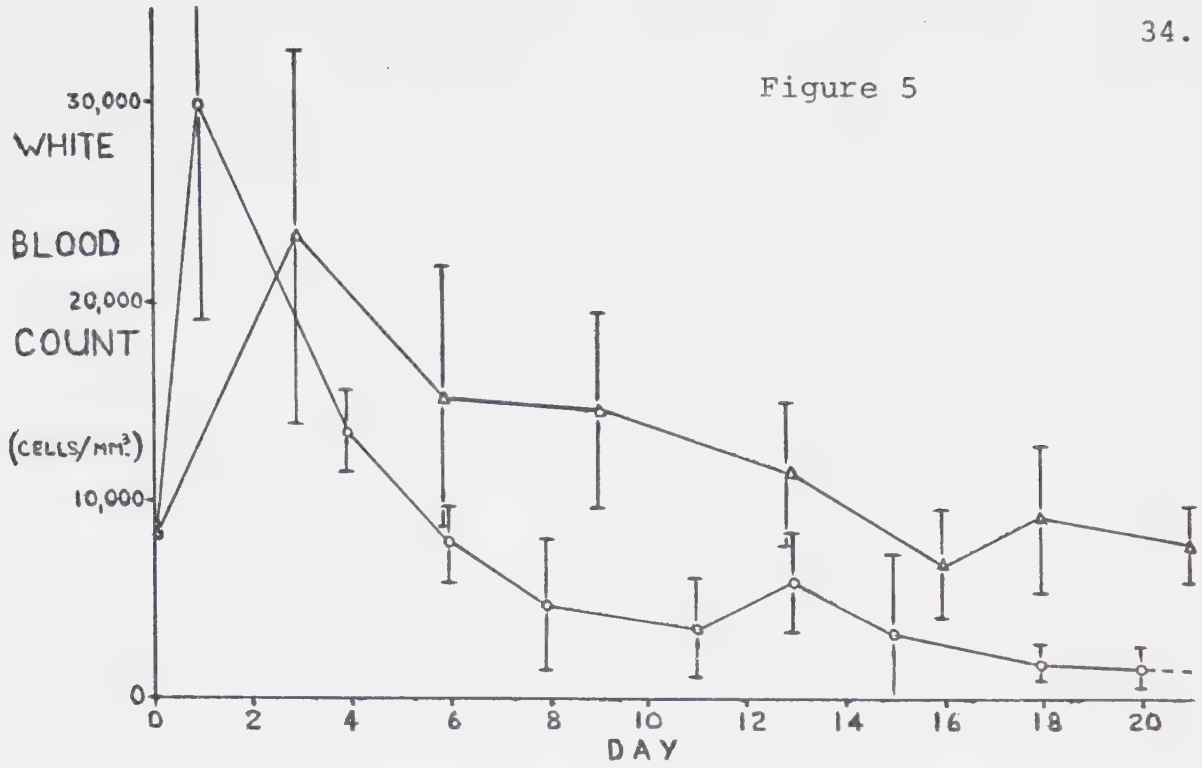
Individual Creatinines - Group 2

Figure 4



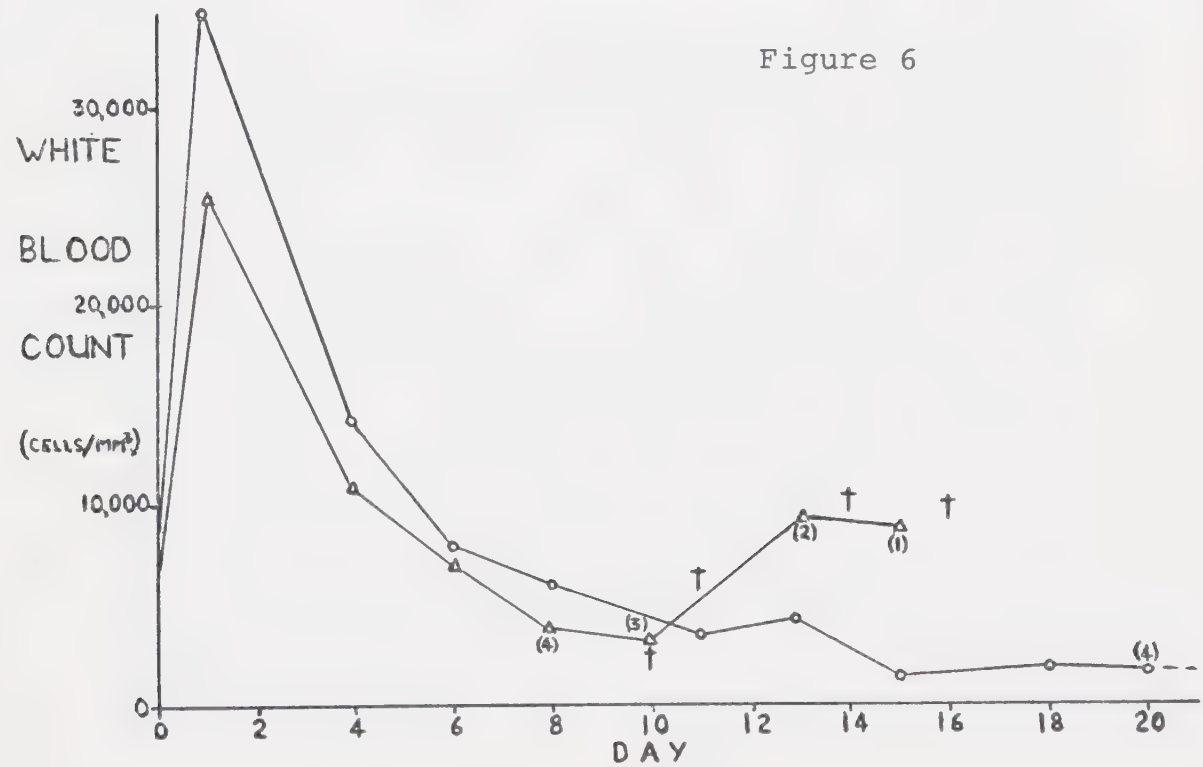
Individual Creatinines - Group 5

Figure 5



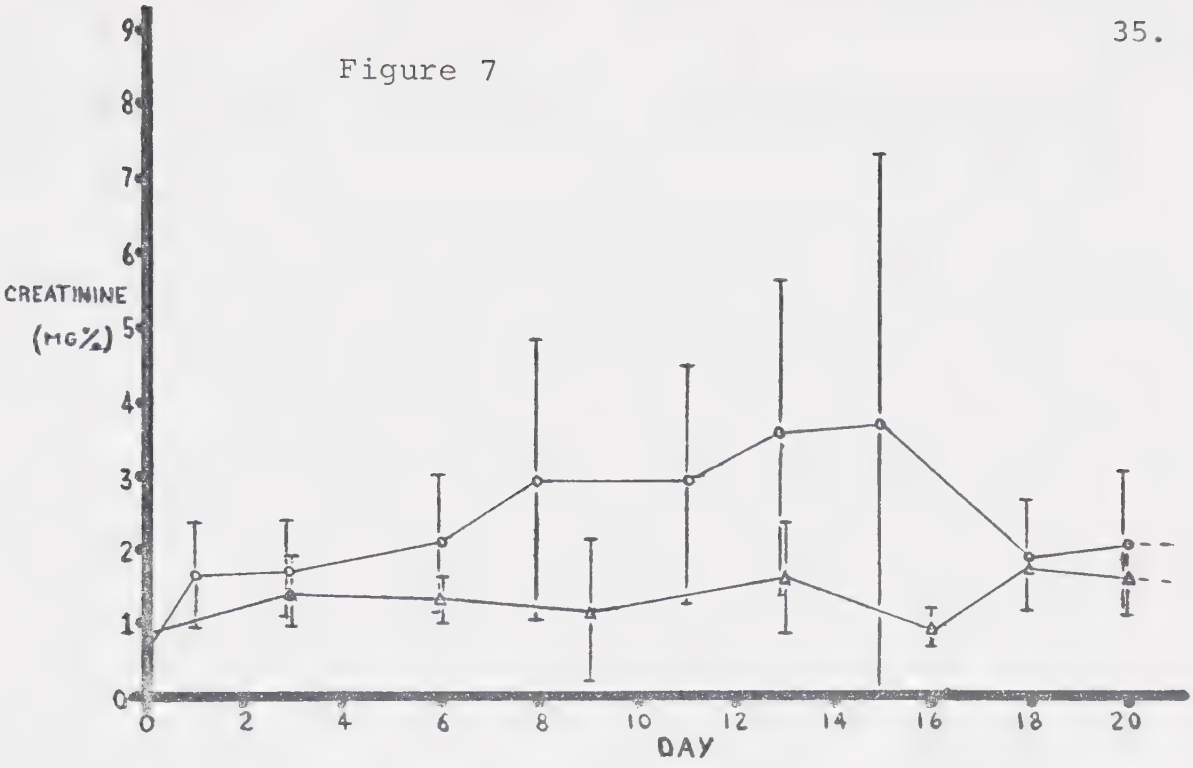
Mean White Blood Counts ±1 S.D. Group 2(Δ) & Group 5(o)

Figure 6



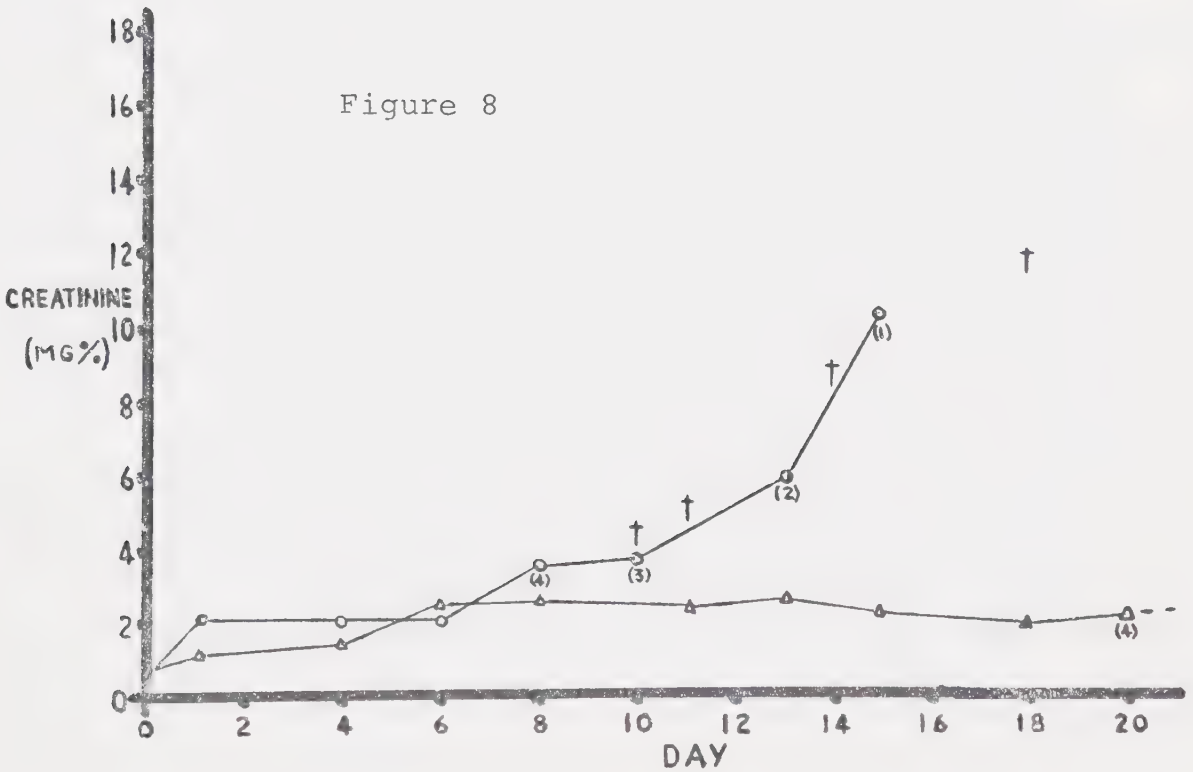
Mean White Blood Counts, Group 5 - 3 week survivors(o) vs. non-survivors(Δ).

Figure 7



Mean Creatinines ±1 S.D. Group 2(o) & Group 5(Δ)

Figure 8



Mean Creatinines, Group 5 - 3 week survivors(Δ) vs. non-survivors(o)

Procaine Penicillin G. following induction of anesthesia, and blood was taken for baseline analysis. An intravenous infusion of 500 to 1000 cc., depending on the dog's weight, of Ringers lactate or 3.3% dextrose and 0.3% Sodium Chloride was given over the time of the procedure.

The abdominal cavities were exposed using full length midline incisions. Following preparation of the site of transplantation (right iliac fossa) in the first recipient, a left nephrectomy was then carried out in the donor. In all instances the left kidney was transplanted unless there were two left renal arteries, in which case the right kidney was used. In one instance of bilateral arterial duplication, a transplant was done using a double arterial anastomosis. The kidneys were not perfused with any solutions at the time of removal. The renal vein was first sutured end to side to the distal inferior vena cava or proximal right iliac vein of the recipient using a continuous circumferential 6-0 silk stitch with two stay sutures. The artery was sutured end to side to the right iliac artery using continuous 6-0 silk stitches between two stay sutures. The vessel clamps were then released. Ischemia time was 25 to 50 minutes. Perfusion of the kidney was usually prompt with good arterial pulsations, kidney tone and colour.

The ureter was then placed in the bladder through

a submucosal tunnel of 2 to 3 cm. in length, the ureteral orifice spatulated and anchored to the upper trigonal border in the midline using three 4-0 chromic stitches.

If a right kidney was used it was placed in the same site but was reversed from its normal orientation so that the lower pole was cephalad. In this way the usual arterio-venous relationship existed (i.e. artery to the recipient's left, vein to the right) but the ureter exited from the pelvis in the wrong direction - toward the recipient's diaphragm. It was then brought around in a slightly redundant loop without tension or kinking and implanted in the bladder in the usual manner.

The reciprocal transplantation was then carried out in a similar manner, followed by contralateral nephrectomies and closure. At some time during the procedure a gastric tube was passed and the initial dose of drugs given in an emulsified solution.

Post-operative Care

The dogs were observed in the operating room until almost awake, then extubated and returned to the Vivarium shortly after. Diet following surgery was variable, but was usually a liquid diet on the first post-operative day, followed by a regular diet. When dogs became anorexic, they were fed whatever they would eat.

Intercurrent disease (e.g. parasites) was treated as it occurred. Seromas and wound infections, which were common in male dogs along the incision lateral to the penis, were treated by skin suture removal in that area and drainage.

Skin sutures were removed at 7 to 10 days post-operatively. Treatment was carried out strictly as indicated earlier with two general exceptions. When the dogs became anorexic, usually due to rejection and terminal uremia, the drugs were not given. This was usually for one or two days before death. Secondly, if the white blood count fell below 2500, Imuran was withheld. This was a problem in the Imuran-Allopurinol group mainly, but because of differences in timing between determination of blood counts and administration of Imuran, several dogs with low blood counts did receive Imuran. Allopurinol was continued throughout. Treatment was stopped at 35 days in this group, as it was felt that this would be a significant prolongation of survival.

At the time of death a full autopsy was done. In addition to a general examination, the kidney, ureters, bladder, and the arterial, venous and ureteric anastomes were carefully examined to determine the presence or absence of thrombosis, obstruction, infection or other complicating factors. Histological sections were taken of

all kidneys and of any other organs that appeared to be abnormal.

Analysis of Results

Three parameters - survival times, serial peripheral total white blood counts and serial serum creatinines - were recorded for analysis. Maximum survival time for statistical purposes was considered to be 40 days, as treatment was stopped in the Allopurinol and Imuran group at 35 days. Mean survival times of the various groups were compared by the "Student" t test.[75]

At the time of surgery, venous blood samples were taken for baseline white blood count and differential, hemoglobin, hematocrit and serum creatinine. Samples were taken post-operatively two or three times weekly for total white blood counts and serum creatinines. Determination of values was done by personnel of the Surgical Medical Research Institute's biochemical laboratory using standard methods.[76,77] As sampling was done on predetermined days of the week, while the surgery was done on various days, the time of sampling in terms of days post-operatively is not the same in all dogs of a series. Accordingly, values were grouped from contiguous days as indicated in the white blood count tables in the appendix before graphic presentation. For example, mean values plotted as being on day 4

may represent a grouping of values from day 3, 4, and 5.

RESULTS

Mean Survivals

Individual survival times are shown on pages 25 to 29. Mean survival of the groups receiving no treatment, Imuran 10mg./kg. M.W.F., or Allopurinol 100mg. t.i.d. were 9.9, 13.3 and 10.2 days respectively. The latter two groups do not differ significantly ($p>0.05$ in both instances) from the untreated group. On the other hand, treatment with a combination of Allopurinol 100mg. t.i.d. and Imuran 10mg./kg. M.W.F. resulted in a mean survival of 23.0 days, significantly greater than the untreated group ($p<0.01$).

Treatment with 5mg./kg. of Imuran daily for three weeks followed by a dose reduction to 5mg./kg. twice weekly resulted in a mean survival of 33.0 days in Group 2. There was one long term survivor in this group. This dog (H564) was treated for approximately three months and then all treament was stopped. It's creatinine was persistently elevated (3 to 5 mg.%) from the second month onward until it died suddenly at nine months. At autopsy no signs of acute disease could be found, but it did have a large thin-walled bladder holding 300-400 cc. of urine, plus a hydronephrosis. The kidney parenchyma was about ½ inch thick. Despite the fact that no bladder outlet obstruction could

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SURVIVAL TIMES

Group 1 - Control - no treatment

Dog	Survival (days)	Comments
H1512	5	Rejection
H1783	8	Artery thrombosed Rejection
H569	10	Rejection
H555	11	Rejection
H308	13	Rejection
H465	12	Rejection
H541	12	Rejection
H553	8	Two intussussceptions. Immediate cause of death - dehiscence. Kidney being rejected

Mean survival (±1 S.D.) = 9.9±2.5

SURVIVAL TIMES

Group 2 - Imuran 5mg./kg. daily

Dog	Survival (days)	Comments
H597	26	Creatinine 1.2 wbc 9,200 Pneumonia
H566	32	Creatinine 2.4 Kidney rejecting. No other abnormalities
H567	27	Rejection Roundworms, coccidiosis treated post-op.
H564	270*	Coccidiosis x 1 mo. post-op Sulfas given.
H44	40	Rejection

Mean survival (±1 S.D.) = 33.0±6.8

*H564 - calculated as 40 day survival

SURVIVAL TIMES

Group 3 - Imuran 10mg./kg. M.W.F.

Dog	Survival (days)	Comments
H1720	15	Rejection
J83	10	Rejection
H661	12	Rejection. Renal Rupture
H634	21	Rejection. Hydroureter, hydro-nephrosis. Possible bladder neck obstruction.
H648	17	Rejection
H573	12	Rejection
H614	6	Probable? rejection.

Mean survival (±1 S.D.) = 13.3±4.9

SURVIVAL TIMES

Group 4 - Allopurinol 100mg. t.i.d.

Dog	Survival (days)	Comments
H1308	6	Dehiscence lower 3" linea alba. Skin intact.
H1415	13	Rejection
H1387	10	Rejection
H1346	10	Rejection
H412	10	Rejection
H712	12	Rejection

Mean survival (±1 S.D.) = 10.2±2.4

SURVIVAL TIMES

Group 5 - Allopurinol 100mg. t.i.d. &
Imuran 10mg./kg. M.W.F.

Dog	Survival (days)	Comments
H1546	41*	wbc 1,700, creatinine 1.0
H1547	29	Rejection
H1557	14	Creatinine 6.5, wbc 9,500 3" intussusception small bowel.
H1566	11	Creatinine 5.2, wbc 800. Kidney uniformly hemorrhagic.
H1517	37	Creatinine 5.2, wbc 4,600
H790	11	Intermittent vomiting post-op. Rejection.
H799	24	wbc 600, creatinine 2.5. Hemorrhage abdominal wall.
H797	18	Rejection

Mean survival (±1 S.D.) = 23.0±11.4

*Calculated as 40 day survival

be demonstrated, it is most likely that the bladder outlet was mechanically obstructed, probably secondary to suturing of the vertical bladder incision used in re-implantation of the ureter.

Pattern of Survival (see figures 3 and 4)

While both Groups 2 and 5 showed significant prolongation of survival, there seemed to be a definite qualitative difference between the two groups.

Of the five dogs receiving Imuran 5mg./kg. daily (Group 2) all had consistently low creatinines during the period of daily treatment (see figure 3 and tables in appendix). At the end of three weeks when the Imuran was given only twice weekly, two dogs (H44 & H567) later showed a rise in creatinine and white cell counts and died as a consequence of rejection. Two others continued with low creatinines but died shortly after, one (H597) with pneumonia and the other (H566) with no cause of death found. Finally H564 became a long term survivor.

There was a much wider range of survival in the group treated with Imuran 10mg./kg. M.W.F. and Allopurinol 100mg. t.i.d. In contrast to the group treated with Imuran 5mg./kg. daily, four out of eight dogs in this group died within the first three weeks, two died later in the

treatment period, and two died after treatment was withdrawn (but not as a consequence of withdrawal - H1517 had a rising creatinine before treatment was stopped, H1546 had a creatinine of 1.0, wbc of 1700 at death on day 41). The cause of death was not always clear cut - several dogs (H1566, H790 & H799) had elevated creatinines and leucopenia, while others (H1547, H1557 & H797) had a normal or elevated w.b.c. plus a rising creatinine. Only one dog (H1546) did not have an elevated creatinine at the time of death - it was also leucopenic (1700).

White Blood Counts and Creatinines

Dogs in the groups given no treatment, Allopurinol 100mg. t.i.d. or Imuran 10mg./kg. M.W.F. all showed a prompt rise in total w.b.c. which usually remained markedly elevated until the time of death. This was accompanied by a progressive rise in serum creatinine.

The individual patterns of survival in the group treated with Imuran 5mg./kg. daily and the group treated with Imuran 10mg./kg. M.W.F. and Allopurinol 100mg. t.i.d. have been described in the previous section. The mean white counts in these groups for the first three weeks are plotted in Figure 5. Both groups showed an initial rise in w.b.c. followed by a return to the normal level in the Imuran daily group, and to a leucopenic level in the Imuran-

Allopurinol group. Despite the lower white counts, four dogs in the latter group died during this period. Despite a rising mean creatinine (Figure 8), the pattern of mean white count for these four dogs is remarkably similar to that of the survivors (Figure 6), both sub-groups (deaths and survivors) being consistently lower than the Imuran daily group with the exception of the last two values for those dogs dying - these values represent two dogs.

The creatinines of the Imuran daily group were consistently lower than those of the Imuran-Allopurinol group, but were always within the range of one standard deviation (Figure 7). Mean creatinines within the Imuran-Allopurinol group were higher in those dogs dying as compared to the survivors during the first three weeks (Figure 8).

DISCUSSION

The mean survival of 9.9±2.5 days in Group 1 is comparable to those obtained in other untreated series[14,78,79]

Group 2 was intended to approximate the more usual schedule of azathioprine therapy - that is, a single daily dose. Such a group would provide a standard to which one could compare the efficacy of any new dose schedule or mode of therapy, as certainly one would not apply a new therapy to general use if it did not provide an advantage over present therapy in terms of survival time or degree of toxicity. The prolongation of survival in this group to 33.0±6.8 days is highly significant when compared to Group 1, and is roughly comparable to Calne's results.[14] The deaths of two out of the five dogs due to causes other than rejection is again similar to Calne's findings of almost 50% mortality due to toxicity and infection, though it is recognized that in a series as small as this, a figure such as two out of five does not carry significance.

The dose schedule of azathioprine 10mg./kg. Monday, Wednesday and Friday in Group 3 was essentially ineffective, though the mean survival of 13.3±4.9 days is slightly longer than that in Group 1, and there are a few individual survivals of greater duration (15, 17 and 21 days). The total weekly dose of 30mg./kg. is close to that of 35mg./kg.

given in Group 2, but with a much less effective result. The main difference between the groups is in the timing of the doses. If the data presented earlier on the timing of azathioprine metabolism and excretion can be applied to the dog, it would seem likely that with an interval of 48 or 72 hours between doses, there is probably a fairly long period during which no azathioprine metabolites are present. The data on timing of azathioprine therapy, as summarized by Schwartz,[80] indicates that azathioprine acts on an early phase of the immune response, at a recognition or inductive step, and is ineffective once the proliferation of antigen sensitive cells has begun. It would therefore seem most reasonable to hypothesize that in this group, the duration between doses was such that the azathioprine was metabolized and excreted, leaving an interval before the next dose during which recognition of the allograft as being immunologically foreign could take place resulting in a response which would progress far enough that it would not be affected by the next dose.

The mean survival of Group 4, 10.2±2.4 days, was not significantly different from the untreated group. Allopurinol 100mg. t.i.d. is therefore not immunosuppressive in the dog. Most probably, allopurinol does not have immunosuppressive properties, but no other data was found in the literature. Elion showed that it was ineffective

as an anti-tumor agent over a wide dose range in the mouse.

The mean survival of 23.0±11.4 days in Group 5 is significantly longer than in Group 1. The two drugs used, allopurinol 100mg. t.i.d. and azathioprine 10mg./kg. M.W.F. were ineffective when used alone. In Elion's study, she showed that allopurinol inhibited 6-mercaptopurine degradation via xanthine oxidase inhibition resulting in a three-fold increase in urinary 6-mercaptopurine excretion and a six to nine-fold decrease in urinary thiouric acid in the mouse.[25] Similarly, in man, urinary 6-MP increased from less than 7.0% to 29% of the oral dose, and thiouric acid decreased from 25.5% down to 3.4% with allopurinol 300mg. and 6-mercaptopurine 150mg.[25] Vogler studied urinary 6-MP only in six leukemic patients, with similar results.[53] As 6-mercaptopurine is a principle product of azathioprine, this degradation block probably occurred in our series, resulting in a greater amount of 6-mercaptopurine being available to enter into the pathways of purine metabolism, and/or a more prolonged presence of 6-MP before excretion.

Whether other interactions exist is unanswerable. It is possible that allopurinol might have immunosuppressive properties not evident at the dose used in Group 4, but the question does not seem to have been investigated. The formation of allopurinol nucleotide with the resulting inhibition of purine metabolism might provide a point of

further interaction with 6-mercaptopurine, but as stated previously the significance of this effect is unknown and probably minimal. Whether it would assume significance when 6-mercaptopurine or azathioprine are also used is a matter of speculation.

Rejection despite leucopenia occurred in several individual cases and in the group as a whole (figure 4 and 6). This has been noted in individual cases previously, both experimentally[15] and clinically,[18] leading to the supposition that the leucotoxic and immunosuppressive effects of azathioprine are separate phenomena.[81] Furthermore, Swanson has shown that in most patients leucopenia is not necessary to attain immunosuppression with azathioprine[56] and J.F. Bach has shown that in vitro immunosuppression occurs at levels much lower than cytotoxic levels.[82] The present experimental results support the contention that the immunosuppressive and leucotoxic effects are, at least in part, separate phenomena.

The therapy in Group 5 was much less satisfactory than with daily azathioprine therapy. There was a much greater survival range with a greater variability of response, and a high incidence of severe leucotoxicity. In addition, while no gross liver abnormalities were noted on autopsy, microscopic sections were not done, so it is possible that hepatotoxicity was also present. This

particular dose schedule offers no advantages over daily azathioprine therapy and several disadvantages. The use of allopurinol in combination with azathioprine for transplantation or in other instances (e.g. patients on azathioprine therapy with gout) appears to be too hazardous to be justified.

Finally, while leucotoxicity was greater with allopurinol in these experiments, in contrast to Elion's results, it may be the experimental design used that may be partially responsible. Our approach was to use a larger but less frequent than usual dose of azathioprine, with the addition of allopurinol, while Elion did not alter the dose timing, but instead showed a decrease in the minimal dose of azathioprine that was effective with the addition of allopurinol. A similar approach in these experiments would be to use a lower daily dose of azathioprine that is presumably ineffective alone (e.g. 2mg./kg./day) plus allopurinol - i.e. azathioprine 2mg./kg./day plus allopurinol 100mg. t.i.d.

CONCLUSIONS

In renal transplantation in mongrel dogs:

A positive interaction between allopurinol and azathioprine exists when used as immunosuppressants which is probably due to potentiation of the immunosuppressive effect of azathioprine by allopurinol.

Allopurinol probably also potentiates leucotoxicity to azathioprine, indicating that clinical use of these two drugs in combination could be hazardous.

Secondarily:

i) Imuran 5mg./kg./day is an effective immunosuppressive regime.

ii) Imuran 10mg./kg. Monday, Wednesday and Friday (M.W.F.) is an ineffective immunosuppressive regime.

iii) Allopurinol 100mg. t.i.d. has no immunosuppressive effect.

iv) A combination of Imuran 10mg./kg. M.W.F. and allopurinol 100mg. t.i.d. is an effective immunosuppressive regime, but offers no advantages over Imuran 5mg./kg. daily.

v) Rejection may occur despite leucopenia.

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APPENDIX

CREATININES (mg%)

Group 1 - Control - No treatment

Dog	H1512	H1783	H569	H555	H318	H465	H541	H553	Av.	S.D.
Day										
0	0.8	0.8	0.7	0.9	0.8	0.9	1.4	1.7	1.0	0.4
1										
2	4.5	3.1							3.8	1.0
3					4.8	3.5	5.2	4.3		
4	11.8	7.3	1.6	5.2						
5	X									
6		↑	↑	↑	6.7	7.8	5.6	11.3	8.8	4.6
7		18.1	5.1	7.2						
8		X						X		
9					8.4	11.1			9.8	1.9
10			X							
11				X			10			
12						X	X			
13					18.7X					
14										
15										
16										
17										
18										
19										
20										

X = day of death

↑↓- moved to that day for purposes of calculation

CREATININES (mg%)

Group 2 - Imuran 5mg./kg. daily

Dog	H564	H597	H566	H567	H44	Av.	S.D.
Day							
0	0.7	1.4	1.2	0.7	0.6	0.9	0.4
1							
2					1.4 ↓		
3	1.7	↑	↑	0.9		1.4	0.4
4		1.8	1.2				
5							
6	1.6	↑	↑	0.9	1.2	1.3	0.3
7		1.6	1.2				
8							
9	0.6			0.5	↑	1.1	1.0
10					2.2		
11							
12		2.9	2.7				
13	1.5	↓	↓	1.3	1.1	1.9	0.8
14		2.3	3.2				
15							
16	0.8			0.8	1.1	0.9	0.2
17							
18		1.7	1.7			1.7	0
19							
20					2.2	1.6	0.5

... continued

CREATININES (mg%)

Group 2 - Imuran 5mg./kg. daily (continued)

Dog	H564	H597	H566	H567	H44	Av.	S.D.
Day							
21	0.8	1.6	1.5	1.8			
22							
23	0.6			6.9	1.3		
24							
25							
26		X	1.4				
27	0.7			X			
28			2.4		2.1		
29							
30	0.8				3.4		
31							
32			X				
33							
34	0.9				11.0		
35							
36							
37	1.4				14.8		
38							
39							
40					X		

X = day of death (H564 - day 270 approx.)

↑↓- moved to that day for purposes of calculation

CREATININES (mg%)

Group 3 - Imuran 10mg./kg. M.W.F.

Dog	H1720	J83	H661	H634	H648	H573	H614	Av.	S.D.
Day									
0	0.9	0.9	0.7	0.9	0.8	0.9	0.5	0.8	0.2
1									
2	7.0	1.3	1.3	1.5	1.1	0.5	3.3	2.2	2.3
3									
4									
5	8.1	1.8							
6	↓	↓	1.6			7.4	9.9X	5.8	3.8
7	7.4	6.7		2.1	2.7			4.7	2.7
8									
9	11.4	12.7	7.6	3.0	7.6	14.1		9.4	4.1
10		X							
11									
12			X			X			
13	15.0			4.8	16.7			12.2	6.4
14									
15	X								
16				8.4	14.4			11.4	4.2
17					X				
18									
19									
20				X					

X = day of death

↑↓- moved to that day for purposes of calculation

CREATININES (mg%)

Group 4 - Allopurinol 100mg. t.i.d.

Dog	H1308	H1415	H1387	H1346	H41	H712	Av.	S.D.
Day								
0	1.1	0.7	1.0	0.9	0.9	0.9	0.9	0.1
1	3.6 ↓	1.5 ↓						
2			↑	↑	1.2	1.3	2.8	2.2
3			2.1	7.0				
4	13.0 ↓	6.5 ↓						
5			4.0	12.5	2.2	1.4	6.6	5.1
6	X							
7			13.0	26.8	8.2	5.2	13.3	9.6
8		8.9 ↓						
9					13.0	9.8	10.6	2.2
10			X	X	X			
11		21.5 ↓						
12						17.4X	19.5	2.9
13		X						
14								
15								
16								
17								
18								
19								
20								

X = day of death

↑↓- moved to that day for purposes of calculation

CREATININES (mg%)

Group 5 - Allopurinol 100mg. t.i.d. & Imuran 10mg./kg. M.W.F.

Dog	H1546	H1547	H1557	H1566	H1517	H790	H799	H797	Av.	S.D.
Day										
0	0.7	0.9	0.8	0.8	0.9	0.8	0.8	0.9	0.8	0.1
1	0.8	1.2	2.8	2.5		1.8	1.2	1.0	1.6	0.8
2										
3	↑	↑	2.8	2.3	2.3	↑	1.3	1.0	1.7	0.7
4	0.7	1.4				1.9				
5										
6	0.7	2.3	2.1	1.6	2.4	3.0	3.5	1.4	2.1	0.9
7										
8	0.7	2.3	4.1	1.3	2.2	6.4	4.6	1.6	2.9	1.9
9										
10			4.3	5.2	3.1					
11	0.7	2.6	↓	↓ X	↓	X	2.8	1.3	2.9	1.6
12										
13	0.6	2.5	6.5		4.0		2.7	5.4	3.6	2.1
14			X							
15	0.8	2.3			2.5		2.8	10.3	3.7	3.7
16					↓					
17					2.5					
18	0.8	2.1			↓		2.1	X	1.9	0.8
19					2.5					
20	0.6	2.9					2.2		2.1	1.0

... continued

CREATININES (mg%)

Group 5 - Allopurinol 100mg. t.i.d. &
Imuran 10mg./kg. M.W.F. (continued)

Dog	H1546	H1547	H1557	H1566	H1517	H790	H799	H797	Av.	S.D.
Day										
21										
22	0.8	3.8			2.0		2.5			
23										
24					2.0		X			
25	0.9	12.4								
26										
27	0.5	14.3			1.2					
28										
29	0.7	X			2.3					
30										
31					2.4					
32	0.8									
33										
34	0.7				5.5					
35										
36	0.8				7.4					
37					X					
38										
39	1.0									
40										

X = day of death

↑↓- moved to that day for purposes of calculation

CREATININES (mg%)

Death in Three Weeks

Group 5 - Allopurinol 100mg. t.i.d. & Imuran 10mg./kg. M.W.F.

Dog	H1557	H1566	H790	H797	Av.	S.D.
Day						
0	0.8	0.8	0.8	0.9	0.8	0.0
1	2.8	2.5	1.8	1.0	2.0	0.8
2						
3	2.8	2.3				
4	↓	↓	1.9	1.0	2.0	0.7
5						
6	2.1	1.6	3.0	1.4	2.0	0.7
7						
8	4.1	1.3	6.4	1.6	3.4	2.4
9						
10	4.3	5.2	X	↑	3.6	2.0
11		X		1.3		
12						
13	6.5			5.4	5.9	0.8
14	X					
15				10.3	10.3	
16						
17						
18				X		
19						
20						

X = day of death

↑↓- moved to that day for purposes of calculation

CREATININES (mg%)

Three Week Survivors

Group 5 - Allopurinol 100mg. t.i.d. & Imuran 10mg./kg. M.W.F.

Dog	H1546	H1547	H1517	H799	Av.	S.D.
Day						
0	0.7	0.9	0.9	0.8	0.8	0.1
1	0.8	1.2		1.2	1.1	0.2
2						
3			2.3			
4	0.7	1.4	↓	1.3	1.4	0.7
5						
6	0.7	2.3	2.4	3.5	2.2	1.2
7						
8	0.7	2.3	2.2	4.6	2.5	1.6
9						
10			3.1			
11	0.7	2.6	↓	2.8	2.3	1.1
12						
13	0.6	2.5	4.0	2.7	2.5	1.4
14						
15	0.8	2.3	2.5	2.8	2.1	0.9
16			↓			
17			2.5			
18	0.8	2.1		2.1	1.9	0.7
19						
20	0.6	2.9	2.5	2.2	2.1	1.0

↑↓- moved to that day for purposes of calculation

WHITE BLOOD CELL COUNTS (/cu.mm.)

Group 1 - Control - No treatment

Dog	H1512	H1783	H569	H555	H308	H465	H541	H553	Av.	S.D.
Day										
0	8850	12300	15000	10300	6700	9150	5600	5700	9200	3295
1										
2	20450	25250							22850	3394
3	↑	↑	↑	↑	16650	23500	15000	7300	19138	6671
4	21300	27350	16000	26000						
5	X									
6		↑	↑	↑	24000	21000	20000	21000	20579	5164
7		26350	9950	21750						
8		X						X		
9					27000	44000			35500	12020
10			X							
11				X			12300			
12						X	X			
13					33000X					
14										
15										
16										
17										
18										
19										
20										

X = day of death

↑↓- moved to that day for purposes of calculation

WHITE BLOOD CELL COUNTS (/cu.mm.)

Group 2 - Imuran 5mg./kg. daily

Dog	H564	H597	H566	H567	H44	Av.	S.D.
Day							
0	12300	2100	3550	12700	9100	7950	4908
1							
2					25000 ↓		
3	15000	↑	↑	26000		23380	9606
4		37300	13600				
5							
6	6500	↑	↑	17000	9800	15210	6792
7		21050	21700				
8							
9	9950			20150	↑	14700	5135
10					14000		
11							
12		17000	9600				
13	8500	↓	↓	14150	8700	11590	3797
14		18600					
15							
16	10100			4600	6700	7133	2775
17							
18		11650	6350			9000	3747
19							
20					6600		

... continued

WHITE BLOOD CELL COUNTS (/cu.mm.)

Group 2 - Imuran 5mg./kg. daily (continued)

Dog	H564	H597	H566	H567	H44	Av.	S.D.
Day							
21	6250	9200	10050	5650		7550	1947
22							
23	4350			15000	5850		
24							
25							
26		X	7850				
27	11400			X			
28			12450		2600		
29							
30	5350				3250		
31							
32			X				
33							
34	21650				5300		
35							
36							
37	24000				5050		
38							
39							
40							

X = day of death (H564 - day 270 approx.)

↑↓- moved to that day for purposes of calculation

WHITE BLOOD CELL COUNTS (/cu.mm.)

Group 3 - Imuran 10mg./kg. M.W.F.

Dog	H1720	J83	H661	H634	H648	H573	H614	Av.	S.D.
Day									
0	12350	8650	15800	11100	9400	16800	7100	11600	3635
1									
2	26800	16050	45450	20650	35800	31700	31300	29679	9733
3									
4									
5	20150	21050							
6	↓	↓	31850			20800	7500X	20270	8632
7	22450	19500		24400	15000			20337	4088
8									
9	30450	30450	42600	18450	36750	32400		31850	8041
10		X							
11									
12			X			X			
13	19450			12300	32500			21417	10242
14									
15									
16				10600	5650			8125	3500
17					X				
18									
19									
20									
21				X					

X = day of death

↑↓- moved to that day for purposes of calculation

WHITE BLOOD CELL COUNTS (/cu.mm.)

Group 4 - Allopurinol 100mg. t.i.d.

Dog	H1308	H1415	H1387	H1346	H41	H712	Av.	S.D.
Day								
0	9600	5000	6150	16850	9400	9950	9492	4140
1	28050 ↓	31050 ↓						
2			↑	↑	28250	25650	26116	4447
3			18000	25700				
4	17100 ↓	10800 ↓						
5			12600	14150	20900	35150	18450	8921
6	X	16000 ↓						
7			21500	14400	24000	29350	21050	6072
8		19450 ↓						
9					27700	25100	24083	4217
10			X	X	X			
11		39800 ↓						
12						33100X	36450	4737
13		X						
14								
15								
16								
17								
18								
19								
20								

X = day of death

↑↓- moved to that day for purposes of calculation

WHITE BLOOD CELL COUNTS (/cu.mm.)

Group 5 - Allopurinol 100mg. t.i.d. & Imuran 10mg./kg. M.W.F.

Dog	H1546	H1547	H1557	H1566	H1517	H790	H799	H797	Av.	S.D.
Day										
0	7350	7600	9200	9150	13650	4250	6450	5750	7925	2847
1	20650	50800	29750	30700		13850	25250	27650	29807	10971
2										
3					15100					
4	14400	12900			↓	12150	16650	10350	13590	2251
5										
6	9700	4400	5200	6600	8800	9700	10550	6750	7713	2286
7										
8			9350	1400	7850	4200	4550	1000	4725	3358
9										
10			2300	800	4050					
11	3250	2300	↓	↓ X	↓	X	4600	6750	3435	1926
12	5350	2750								
13	3700	4250	9450		5400		4750	9700	6208	2668
14			X							
15	2200	1250					400	9200	3263	4026
16										
17					3350					
18	1250	1350			↓		1700	X	1913	977
19										
20	1000	2200			2950		850		1750	1002

... continued

WHITE BLOOD CELL COUNTS (/cu.mm.)

Group 5 - Allopurinol 100mg. t.i.d. &
Imuran 10mg./kg. M.W.F. (continued)

Dog	H1546	H1547	H1557	H1566	H1517	H790	H799	H797	Av.	S.D.
Day										
21										
22	2750	5050			2100		600			
23										
24					2800		X			
25	5000	14550								
26										
27	5000	13050			4350					
28										
29	4150	X			3300					
30										
31					2800					
32	2300									
33										
34	1700				1200					
35										
36	1450				4600					
37					X					
38										
39	1700									
40										

X = day of death

↑↓- moved to that day for purposes of calculation

WHITE BLOOD CELL COUNTS (/cu.mm.)

Deaths in Three Weeks

Group 5 - Allopurinol 100mg. t.i.d. & Imuran 10mg./kg. M.W.F.

Dog	H1557	H1566	H790	H797	Av.	S.D.
Day						
0	9200	9150	4250	5750	7087	2487
1	29750	30700	13850	27650	25487	7862
2						
3						
4			12150	10350	11250	1272
5						
6	5200	6600	9700	6750	7062	1891
7						
8	9350	1400	4200	1000	3987	3848
9						
10	2300	800	X	↑	3283	3094
11		X		6750		
12						
13	9450			9700	9575	177
14	X					
15				9200	9200	
16				X		
17						
18						
19						
20						

X = day of death

↑↓- moved to that day for purposes of calculation

WHITE BLOOD CELL COUNTS (/cu.mm.)

Three Week Survivors

Group 5 - Allopurinol 100mg. t.i.d. & Imuran 10mg./kg. M.W.F.

Dog	H1546	H1547	H1517	H799	Av.	S.D.
Day						
0	7350	7600	13650	6450	8762	3295
1	30650	50800		25250	35366	13465
2						
3			15100			
4	14400	12900	↓	16650	14762	1557
5						
6	9700	4400	8800	10550	8362	2736
7						
8			7850	4550	6200	2333
9						
10			4050			
11	3250	2300	↓	4600	3550	1000
12	5350	2750				
13	3700	4250	5400	4750	4525	724
14						
15	2200	1250		400	1283	900
16						
17						
18	1250	1350	3350	1700	1912	977
19						
20	1000	2200	2950	850	1750	1002

↑↓- moved to that day for purposes of calculation